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ANALYSIS OF PSYCHOTROPIC COMPOUNDS IN FUNGI OF THE GENUS PSILOCYBE BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

High-performance liquid chromatography (HPLC) was used for the analysis of the minor consistuents psilocybin and psilocin in fungi of the genus Psilocybe. The separation and determination of these compounds was carried out on a stationary phase of LiChrosorb RP-18. The analytical column (A) and semipreparative column (B) were eluted isocratically with water–ethanol–acetic acid (79.2:20:0.8) at flow-rates of 20 ml/h (A) and 180 ml/h (B). The compounds were detected with a UV detector at 267 nm and a fluorometric detector (excitation, 280 nm; emission, 360 nm). The UV detection limit of psilocybin was 20–40 ng (267 nm) and several ng could be detected fluorometrically. The identity of the compounds was verified by HPLC and thin-layer chromatography and by mass spectrometry and UV spectroscopy. The compounds were determined by means of a direct calibration method and by means of the method of internal normalization. The standard deviation of the determination was ±3.4% (relative). The above methods were used to determine these compounds in extracts of fruit bodies of two species of the genus Psilocybe growing at various places in Czechoslovakia, and found to contain 0.25–1.15% of psilocybin and 0.02–0.16% psilocin per dry mass.

INTRODUCTION

Psychotropic compounds of the indole type are present only at low concentrations in natural materials. Fungi of the genus Psilocybe contain minor quantities of compounds of the tryptamine type, *e.g.*, psilocybin [3-(2-dimethylaminoethyl)indol-4-ol dihydrogen phosphate ester], its dephosphorylated derivative psilocin and monomethyl derivative baeocystin, which exhibit hallucinogenic properties.

The biological and physiological significance of these compounds is receiving great attention at present, primarily from biologists and physicians, even 25 years after the discovery of the compounds in the fungus *Psilocybe mexicana*[1–3]. In 1973, Semerdžieva and Nerud[4] analyzed a methanolic extract of the fungi *Psilocybe semilanceata* (Fr.) Kumm, and *Psilocybe coprinifacies* (Roll.), growing in Czechoslovakia by means of thin-layer chromatography (TLC).

In 1979, White[5] analyzed an extract from the fungus *P. semilanceata* by means

of high-performance liquid chromatography (HPLC) on silica gel eluted with a mixture of methanol, water and 1 *M* ammonium nitrate buffered to pH 9.7. The presence of psilocybin and psilocin was revealed by UV detection (254 nm) and baeocystin by mass spectrometry. Perekal *et al.*[6] using the same method determined psilocybin and psilocin on a chromatographic column packed with ion exchanger and eluted with a mixture of methanol, water, 0.2% ammonium phosphate and 0.1% sodium chloride. In eight samples of *P. subaeruginoase* they determined 0.01–0.2% of psilocybin using a UV detector (267 nm) and a fluorescence detector (excitation, 267 nm; emission, 335 nm). Christiansen *et al.*[7] detected psilocybin in Norwegian *P. semilanceata* using HPLC on silica gel eluted with a similar mobile phase of methanol, water and 1 *M* ammonium nitrate. With the aid of a UV detector (254 nm) and a fluorescence detector (excitation, 267 nm; emission, 335 nm) they analyzed five samples which were found to contain 0.2–2% of psilocybin. Beug and Bigwood[8] used ion-pair HPLC with methanol, water, acetonitrile and the supplementary agents (PIC-B-7) and (PIC-A) Waters as a mobile phase for the determination of psilocybin and psilocin. When analyzing extracts of the fungus *P. baeocystis* they found 2.0 ± 0.2 mg and 1.3 ± 0.2 mg of psilocybin and psilocin, respectively, per g dry fungus.

It was the aim of the present work to develop a rapid method yielding accurate results which could be used for the determination of psychotropic compounds in fungi of the genus Psilocybe growing in Czechoslovakia.

EXPERIMENTAL

Chemicals

Ethanol was of UV grade, acetic acid and *n*-butanol were of analytical grade. Twice distilled water was used as mobile phase, methanol for UV was employed for the extraction. Hydrochloric acid (Lachema, Brno, Czechoslovakia) and *p*-dimethylaminobenzaldehyde (Research Institute for Organic Synthesis, Rybitví, Czechoslovakia) were used for the detection in TLC. TLC foils Silufol (Kavalier, Votice, Czechoslovakia) were used. Standard samples of psilocybin and psilocin were of highest purity (Sandoz, Basle, Switzerland).

Microbiological material

Two species of fungi of the genus Psilocybe, *i.e.*, *Psilocybe semilanceata* (Fr.) Kumm. and *Psilocybe bohemica* Šebek were collected at various places in Czechoslovakia over the period 1969–1982. More than twenty samples, comprising mostly dry and fresh fruit bodies and several samples of caps and stems, were analyzed.

Extraction

A 300-mg amount of dry or 3 g of fresh, accurately weighed fruit bodies of the above mentioned fungi were homogenized for 2 min with 30 ml of methanol in a 50-ml flask (20,000 rpm). The suspension was extracted on a reciprocal shaker (amplitude 7 cm) for 16 h at 25°C and then filtered. The methanolic extract was evaporated under vacuum to dryness (30°C) and the residue dissolved in 3 ml of methanol. This solution was used for both HPLC and TLC.

High-performance liquid chromatography

The separation and determination of psilocybin and psilocin was performed in the apparatus Varian LC 8500 on an analytical column (A, 2 × 250 mm) packed with LiChrosorb RP-18 (5 μm) and on a semipreparative column (B, 8 × 500 mm) packed with LiChrosorb RP-18 (10 μm). The columns were eluted isocratically with ethanol–water–acetic acid (20:79.2:0.8) at flow-rates of 20 ml/h (A) and 180 ml/h (B). Column temperature: 25°C. Column pressure: 34 MPa (A); 23 MPa (B).

A variable wavelength UV spectrometer Variscan with a flow-through cell (8 μl) operated at 267 nm and a fluorometric detector Varian Fluorichrom (excitation, 280 nm; emission, 360 nm) were used for the detection.

Sample doses were 1–5 μl of methanolic solution (concentration about 1 mg/ml) in the analytical column and 50–100 μl in the semipreparative column, respectively.

The samples of 100 μl were repeatedly injected on the semipreparative column and the fraction containing the analyzed compound was collected in a 5-ml flask during the elution. It was then evaporated under vacuum and the residue was used for identification by mass spectrometry (MS) and ultraviolet (UV) spectroscopy. Standard compounds and samples were applied to TLC foils as 0.1-ml aliquots (about 20 μg of the compound).

Qualitative analysis of psychotropic compounds was performed by comparing their elution volumes with those of reference samples. The results were confirmed chromatographically by TLC and MS and UV spectroscopy.

For comparison, within a concentration range of 10^{-6}–10^{-7} g/l, the direct absolute calibration technique was used. The reproducibility of the results was determined by means of the method of internal normalization and evaluated according to standard deviation. Chromatograms were recorded on a line recorder Varian A 25 and evaluated with the integrator Varian CDS 111.

Thin-layer chromatography

Psilocybin and psilocin were identified on Silufol foils eluted with *n*-butanol–acetic acid–water (24:10:10)[9]. The psychotropic compounds were detected with the Ehrlich reagent (2% *p*-dimethylaminobenzaldehyde in 1 *M* hydrochloric acid) and yielded red-violet spots after completion of the reaction (psilocybin, R_F = 0.22–0.33; psilocin, R_F = 0.50–0.54)[4,10].

Mass spectrometry

Mass spectra were measured in the apparatus Varian MAT 311. Conditions: ionization energy, 70 eV; current, 1 mA; ion source temperature, 200°C; inlet temperature, 35°C for psilocin and 140°C for psilocybin. The high-resolution peak-matching technique employed gave errors of ± 5 ppm.

RESULTS AND DISCUSSION

In this laboratory, HPLC has been used for some years in the separation and determination of compounds of the indole type[11]. After a previous determination of intermediates of the natural phytohormone —indole-3-acetic acid— we applied the method for the analysis of biologically interesting psychotropic compounds of the tryptamine type found in fungi of the genus Psilocybe.

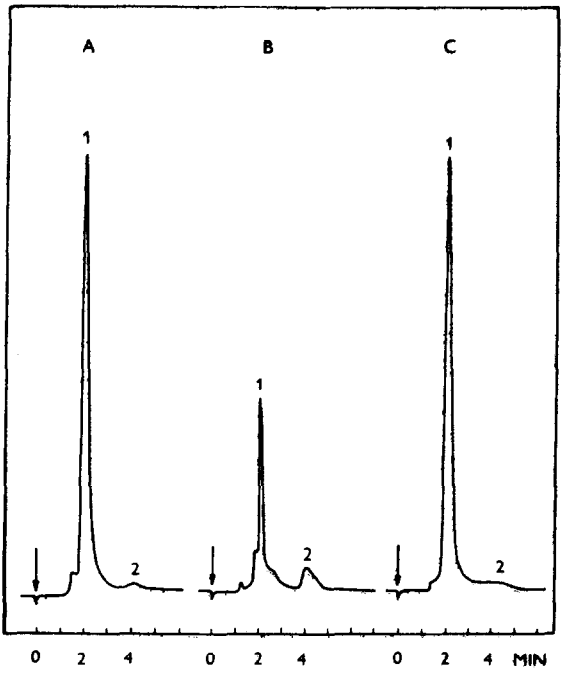

Fig. 1. Chromatograms of the extract of the fungus *Psilocybe bohemica* Šebek. Analytical column: Li-Chrosorb RP-18. Mobile phase: water–ethanol–acetic acid (79.2:20:0.8). Flow-rate: 20 ml/h. Pressure: 34 MPa. Detector: Variscan UV at 267 nm. A, Fresh; B, frozen; C, dry fruit body. Peaks: 1 = psilocybin; 2 = psilocin.

The analysis of psilocybin and psilocin by HPLC was not too challenging with respect to the chromatographic column used. Fig. 1 shows a chromatogram of an extract of the fungus *Psilocybe bohemica* Šebek detected in UV light at 267 nm. Due to its polar character, psilocybin was only weakly retained on the stationary phase (relative retention, $r_{i,s}$ = 1.00; retention volume = 1.12 ml) and psilocin had relative retention $r_{i,s}$ = 1.94. The analysis was based on the chromatographic identification of psilocybin and psilocin in the biological material as the short time of analysis and small elution volumes of these compounds could not serve for their accurate qualitative determination, and on selection of suitable methods used for the determination and evaluation of chromatograms.

The TLC analysis of the extracts and the typical red-violet colour of the psychotropic compounds enabled the identification of these compounds.

Psilocybin was detected by means of MS. The sample was applied more than ten times to the semipreparative column, the mobile phase was evaporated to dryness under vacuum and psilocybin was detected in the residue. Fig. 2A illustrates the mass spectrum of standard psilocybin and Fig. 2B shows that of the compound isolated from sample 8 of *Psilocybe bohemica* Šebek (see Table I). The spectrum of psilocybin is characterized by the ion at *m/z* 58 (C_3H_8N) formed by splitting of the side chain in position β with respect to nitrogen and the ion $(M - H_2PO_3)^+$, $C_{12}H_{16}N_2O$, at *m/z* 204 produced by elimination of H_2PO_3. The spectrum of psilocin is similar: *m/z* 58 (C_3H_8N) and M^+ ($C_{12}H_{16}N_2O$) at *m/z* 204.

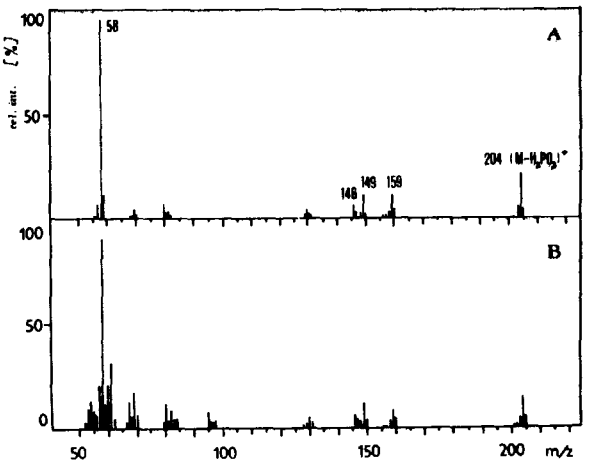

Fig. 2. Mass spectra of an extract of *Psilocybe bohemica* Šebek. A, Psilocybin standard; B, compound isolated from the extract.

TABLE I

CONTENTS OF PSYCHOTROPIC COMPOUNDS IN FRUIT BODIES OF THE GENUS PSILOCYBE

	Species	*Locality, year*	*Parts of fungus*	*Psilocybin (%)**	*Psilocin (%)**
1	*Psilocybe semilanceata* (Fr.) Kumm.	Opava, North Moravia, 1969	Cap Stem	0.74 0.45	0.68 0.04
2	*P. semilanceata*	Rožnov, North Moravia, 1981	Cap Stem	0.83 0.33	0.08 0.10
3	*P. semilanceata*	Krásná Lípa North Bohemia, 1970	Fruit body	0.91	0.09
4	*P. semilanceata*	Prague, Central Bohemia, 1980	Fruit body	1.05	0.12
5	*P. bohemica* Šebek	Stříbrná Skalice, Central Bohemia, 1974	Cap Stem	0.44 0.32	0.05 0.07
6	*P. bohemica*	Sázava, Central Bohemia, 1977	Cap Stem	0.82 0.32	0.04 0.04
7	*P. bohemica*	Blansko, South Moravia, 1980	Cap Stem	0.74 0.54	0.03 0.03
8	*P. bohemica*	Frenštát, North Moravia, 1981	Fruit body	0.46	0.07
9	*P. bohemica*	Sázava, Central Bohemia, 1971	Fruit body	0.25	0.04
10	*P. bohemica*	1974	Fruit body	0.50	0.07
11	*P. bohemica*	1977	Fruit body	0.63	0.06
12	*P. bohemica*	1982	Fruit body	1.14	0.02

* % of dry fruit bodies.

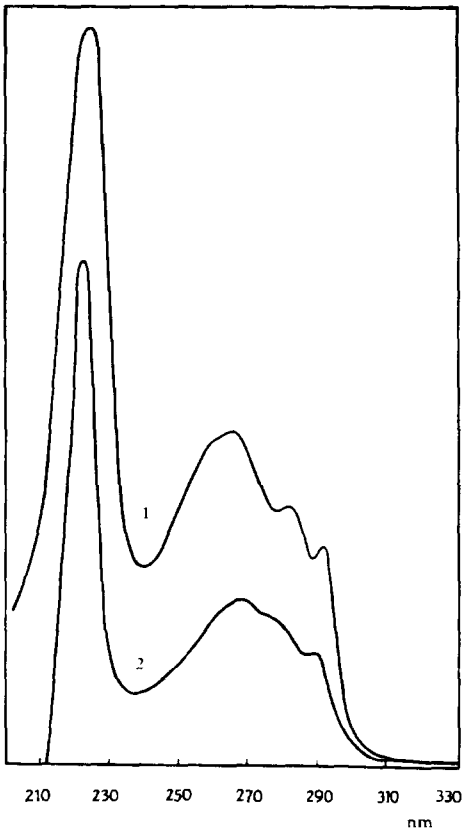

Fig. 3. UV spectra of an extract of *Psilocybe bohemica* Šebek. Curves: 1 = psilocin; 2 = psilocybin.

A portion of the solution of the eluted compound in the mobile phase was used for measurement of the UV spectrum. The spectra of compounds isolated from sample 8 (Fig. 3) fully correspond to literature data: psilocybin, UV max. (methanol) 220, 267, 290 nm; psilocin, UV max. 222, 260, 267, 283, 293 nm[12].

The determination of psilocybin and psilocin by the direct calibration technique and by the internal normalization method yielded fully satisfactory results. With the latter method, the standard deviation from eleven determinations using the integrator did not exceed ±3.4% (relative). The detection limits for psilocybin and psilocin in the mobile phase were 20 and 40 ng, respectively, when measured by the UV detector. The fluorescence detector had a limiting sensitivity about an order of magnitude higher, *i.e.*, ng quantities could be detected.

Methanolic extracts of fruit bodies and various parts of fruit bodies of two species of the genus Psilocybe growing at various localities in Czechoslovakia were analyzed by means of the method described (Table I). It is seen that the two species differ in their psilocybin content; *P. semilanceata* contains more psilocybin than *P. bohemica*. The content of psilocybin in dried fruit bodies of the genus Psilocybe decreases on storage. Caps contain more psilocybin than stems. The content of psilocybin in fruit bodies does not decrease on drying. Boiling of fruit bodies in water

results in a quantitative extraction of psilocybin. The subsequent extraction of fruit bodies with methanol did not yield even traces of psilocybin.

The amount of psilocybin in fruit bodies of the genus Psilocybe found in Czechoslovakia varies from 0.25 to 1.15%. The content of psilocin is negligible, varying within the range of 0.02–0.16% per dry mass.

REFERENCES

1 R. G. Wasson, *Life*, 42 (May 13, 1957) 100–120.
2 V. P. Wasson and R. G. Wasson, *Mushrooms, Russia and History*, Pantheon, New York, 1957.
3 A. Hofmann, R. Heim, A. Brach, H. Kobel, A. Frey, H. Ott, T. Petrzilka and F. Troxler, *Helv. Chim. Acta*, 42 (1959) 2073–2103.
4 M. Semerdžieva and F. Nerud, *Česká Mykologie*, 27 (1973) 42–47.
5 P. C. White, *J. Chromatogr.*, 169 (1979) 453–456.
6 M. Perkal, G. L. Blackman, A. L. Ottrey and L. K. Turner, *J. Chromatogr.*, 196 (1980) 180–184.
7 A. L. Christiansen, K. E. Rasmussen and F. Tonnesen, *J. Chromatogr.*, 210 (1981) 163–167.
8 M. W. Beug and J. Bigwood, *J. Chromatogr.*, 207 (1981) 379–385.
9 J. Picker and W. Richards, *Austr. J. Chem.*, 23 (1970), 853–855.
10 R. Heim, K. Genest, D. W. Hughes and G. Belec, *J. Forensic Sci. Soc.*, 4 (1966) 192–201.
11 M. Wurst, Z. Přikryl and V. Vančura, *J. Chromatogr.*, 191 (1980) 129–136.
12 *The Merck Index*, Merck & Co., Rahway, NY, 9th ed., 1976, p. 1027.